

The Virtual Free Radical School

Redox State and Redox Environment

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Historically

Redox state is the ratio of the interconvertible oxidized and reduced form of a specific redox couple.

Theodor Bücher and Hans Krebs focused on NADH, whose redox state they expressed as the ratio.

$[\text{free NAD}^+]/[\text{free NADH}]$.

Bücher, T.; Klingenberg, M. Wege des Wasserstoffs in der lebendigen Organisation. *Angew. Chem.* **70**:552-570; 1958.

Krebs, H.A. The redox state of nicotinamide adenine dinucleotide in the cytoplasm and mitochondria of rat liver. *Advances in Enzyme Regulation.* **5**:409-434; 1967.

Krebs, H.A.; Gascoyne, T. The redox state of the nicotinamide-adenine dinucleotides in rat liver homogenates. *Biochem. J.* **108**:513-520; 1968.

New Definition of Redox State, which better represents concentration- dependent redox couples (e.g. GSSG/2GSH)

The **redox state** of a redox couple is defined by the half-cell reduction potential and the reducing capacity of that couple.

A convenient notation for the status of a redox pair, such as GSSG/2GSH, would be:

$\{E_{hc}(\text{GSH}); [\text{GSH}]\}$, e.g. $\{-180 \text{ mV}(\text{GSH}); 3.5 \text{ mM}\}$.

Redox Environment

Historically, the term **redox state** has been used to describe specific redox couples. It is inappropriate to use the term redox state with systems that have many linked redox couples, such as cells and tissues. Therefore, we suggest the use of **redox environment** for these systems.

The redox environment of a **linked** set of redox couples (as found in a biological fluid, organelle, cell, or tissue) is the summation of the products of the reduction potential and reducing capacity of the linked redox couples present.

Schafer FQ, Buettner GR. (2001) Redox state of the cell as viewed through the glutathione disulfide/glutathione couple. *Free Radic Biol Med.* **30**:1191-1212.

The Nernst Equation

The voltage of an electrochemical cell is calculated with the Nernst equation.

$$\Delta E = \Delta E^{\circ} - \frac{RT}{nF} \ln Q \qquad Q = \frac{[\text{Ox}_1]^c [\text{Red}_2]^d}{[\text{Red}_1]^a [\text{Ox}_2]^b}$$

where R is the gas constant ($R = 8.314 \text{ J K}^{-1} \text{ mol}^{-1}$), T the temperature (in Kelvin), and F the Faraday constant ($F = 9.6485 \times 10^4 \text{ C mol}^{-1}$).

This will yield results in Volts.

pH is an Important Factor in the Nernst Equation

$$E_{\text{pH}} = E^{\circ'} + ((\text{pH} - 7.0) \times (DE/D\text{pH})) \text{ mV}$$

where $\Delta E/\Delta\text{pH}$ is the change in E if the pH is increased by 1 unit,

For example GSSG/2GSH at 25°C:

$$E_{7.4} = -240 + ((7.4 - 7.0) \times -59.1) \text{ mV} = -264 \text{ mV.}$$

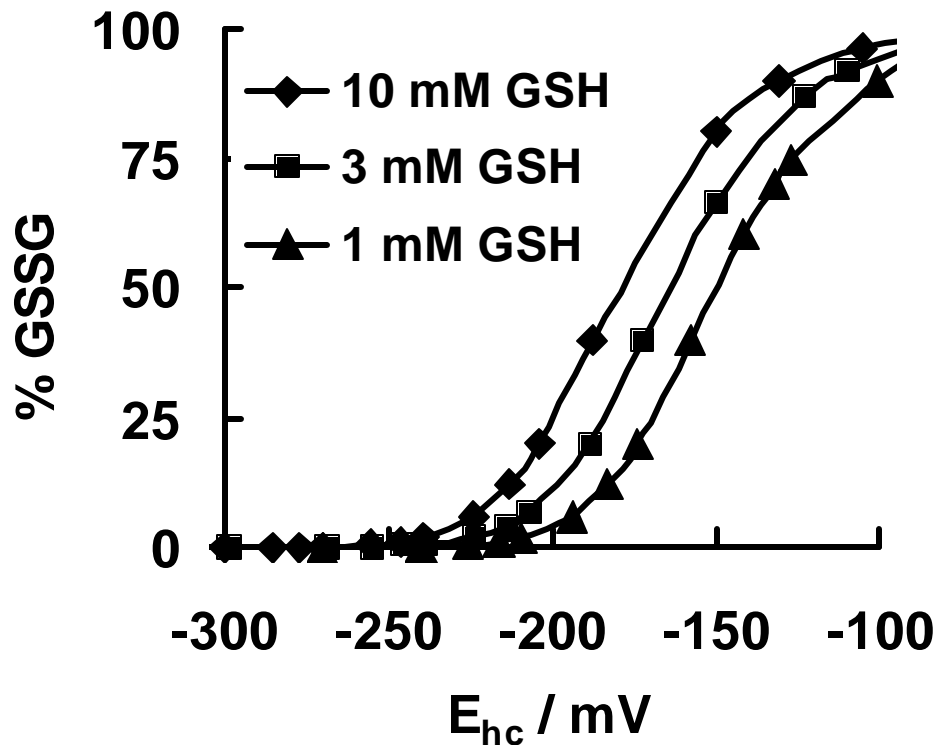
$$E_{7.3} = -240 + ((7.3 - 7.0) \times -59.1) \text{ mV} = -258 \text{ mV.}$$

The value of $\Delta E/\Delta\text{pH}$ is dependent on the number of electrons and the number of protons involved in the process.

The Redox State of Some Redox Couples is Concentration Dependent, e.g GSSG/2GSH



$$E_{\text{hc}} = -240 - (59.1/2) \log ([\text{GSH}]^2/[\text{GSSG}]) \text{ mV at } 25^\circ\text{C, pH } 7.0.$$



The red. potential of the GSSG/2GSH half-cell is dependent on both the ratio of $[\text{GSH}]/[\text{GSSG}]$ and the concentration of GSH.

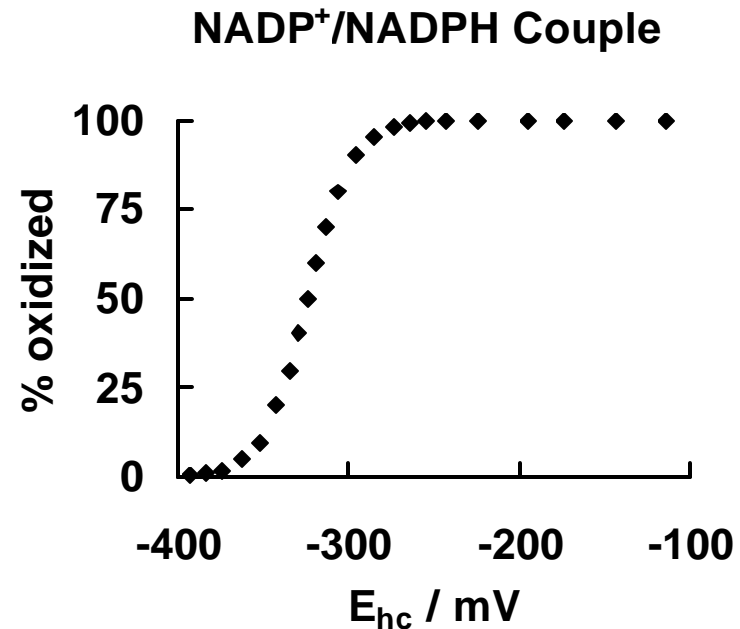
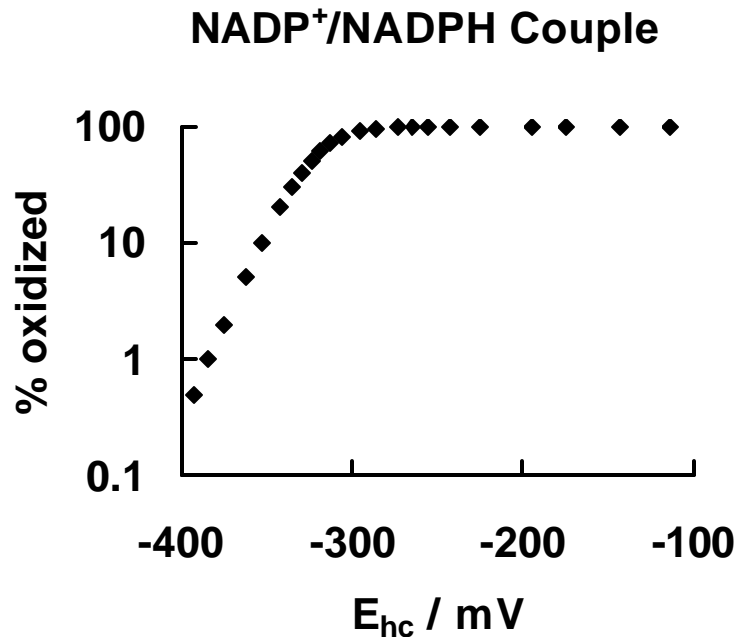
The ordinate represents the percent GSH that has been oxidized to GSSG. The 10, 3 and 1 mM GSH are initial concentrations of GSH before any is oxidized.

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The Redox State of the NADP⁺/NADPH Couple is Not Concentration Dependent

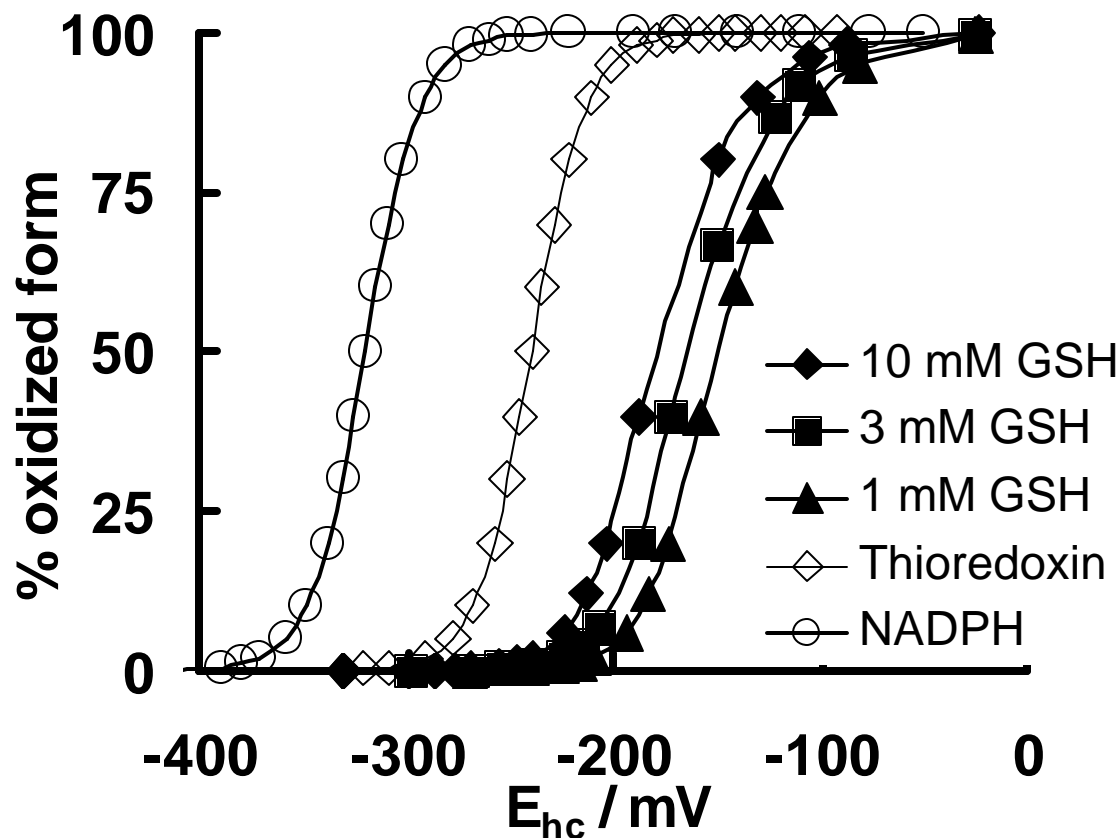


$$E_{\text{hc}} = -315 - (59.1/2) \log ([\text{NADPH}]/[\text{NADP}^+]) \text{ mV at } 25^{\circ}\text{C, pH } 7$$



Schafer FQ, Buettner GR. (2001) Redox state of the cell as viewed through the glutathione disulfide/glutathione couple. *Free Radic Biol Med.* **30**:1191-1212.

Cellular Redox Systems



The $\text{NADP}^+/\text{NADPH}$ couple provides the reducing equivalents needed for the thioredoxin and GSH system.

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Why GSH is Considered the Major Redox Buffer: An Example

If $[GSH] \approx 5 \text{ mM}$, $E_{hc} (GSSG/2GSH) = -240 \text{ mV}$,

$[NADPH] \approx 0.1 \text{ mM}$, and $E_{hc} (NADP^+, H^+/NADPH) = -370 \text{ mV}$,

then

Redox Environment =

$$(5 \text{ mM} \cdot -240 \text{ mV}) + (0.1 \text{ mM} \cdot -370 \text{ mV})$$

$$((-1200) + (-40)) \text{ mV mM} = \mathbf{-1240 \text{ mV mM}}$$

Conclusion

The cellular redox environment results from the composite of various linked redox couples.

The term redox state should be reserved to reference specific redox couples and is best expressed through the use of the Nernst equation.